BACTERICIDIC A[illegible] NITROGEN DIOXIDE ON TH[illegible] VEGETATIVE AND SPOROUS FORMS OF BACILLUS ANTHRACIS

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/Following is a translation of an article by A. A. Polyakov, T. A. Trzhetsetskaya, K. N. Arbuzov, A. A. Akhumova and K. P. Chepurov of the Poltava Agricultural Institute, Microbiology Department, in the Ukrainian-language periodical Mikrobiolohichnyy Zhurnal (Journal of Microbiology), Vol. 24, No. 6, Kiev, 1962, pages 43-45. The article was submitted to the editors on 11 December 1961./

The high mobility, penetrating power, adsorptivity and the highest contact properties of gas molecules of all aggregate states give gaseous bacteriocides an advantage over liquid and especially over powder disinfectants. On this basis we tested various gaseous substances, nitrogen dioxide in particular, as disinfectants of objects contaminated with anthrax bacilli.

The bacteriocidic properties of nitrogen dioxide, as far as we know from literature, have not been investigated by anyone. This gas is not listed in handbooks among disinfectants, insecticides or fungicides used in the destruction of pathogenic microorganisms.

Nitrogen dioxide (NO_2) is a red-brown gas with powerful oxidizing action. Industrially it is obtained by combining atmospheric nitrogen and oxygen or by burning synthetic ammonia in oxygen. It is an intermediate product of chemical plants which synthesize nitric acid and nitrogen fertilizers.

NO_2 is transported as liquid in metal tanks.

We studied the bacteriocidic properties of NO_2 on many microbes -- spore formers and nonspore formers -- which cause disease in humans, animals, and plants (staphilococci, streptococci, spores of tetanus agents,

emphysematous carbuncle, gas gangrene, corn blight, darnel and smut, spores of hay and potato bacilli). It was definitely established that vegetative forms of microbes at the concentration of gas of 0.5 g per liter of air die in 2-3 minutes and sporous forms in 5-10 minutes.

At the All-Union Scientific Research Institute of Veterinary Sanitation (Vsesoyuznyy Naukovo-Doslidnyy Institut Veterynarnoyi Sanitariyi) the Commission then investigated the bacteriocidic action of nitrogen dioxide on vegetative and sporous forms of anthrax.

In these investigations a strain No. 66 of Bac. Anthracis was used, obtained from the museum of microorganisms of cultures of the All-Union Institute of Experimental Veterinary Medicine (Vsesoyuznyy Institut Eksperymental'noyi Veterynariyi). It had typical morphological and cultural properties and was highly virulent.

A suspension of spores of Bac. Anthracis of one billionth concentration infected 32 sterile gauze test objects, which were then placed into two one-liter flasks with 0.5 g of nitrogen dioxide each. In the first bottle, the test objects were kept for 15 min. and in the second, for 30 min. After completion of the exposure indicated for test objects in groups of ten the test objects from each bottle were transferred into test tubes with meat-peptone broth, which were placed into a thermostat. In addition, ten test objects from another bottle were washed with 3 ml of physiological solution and the suspension thus obtained was injected into five mice in doses of 0.2 ml.

For control of the vitality of anthrax spores, with which the test objects were infected one test object was not treated with nitrogen dioxide, but placed into the broth. For verification of the virulence of the starting suspension of spores it was injected into control mice in the same dose of 0.2 ml.

In ten days none of the twenty test tubes with broth in which test objects, previously treated with nitrogen dioxide, had been placed showed growth of Bac. Anthracis, and for that matter, of any other microbes. All mice which were injected with the suspension of anthrax spores treated with nitrogen dioxide remained alive.

In the control test tube a violent growth of Bac. Anthracis was apparent -- the upper layer of the broth remained clear and the bottom of the test tube contained fluffy sediment which was easily dispersed by shaking.

The control mouse which was infected with a suspension of anthrax died in forty-eight hours. The death of the animal from anthrax was verified by microscopic and culture studies.

In the decontamination process of anthrax test objects with nitrogen dioxide an insignificant fraction of this gas could have been adsorbed on their surface and after introduction into culture medium it was dissolved and exerted bacteriocidic action. In such a case the growth of Bac. Anthracis could have been absent not because of the destruction of spores, but because of the unfavorable medium.

To solve this question we ran simultaneously two other experiments.

1) After ten-minute treatment with broth two infected test objects, treated with nitrogen dioxide, were transferred into fresh sterile broth. In fresh media the growth of anthrax bacilli and of other microbes was not observed.

2) We seeded one string of Bac. Anthracis culture in six test tubes of sterile broth containing test objects infected and treated with nitrogen dioxide. The tubes were kept for ten days in a thermostat. Anthrax microbes bred in all six tubes.

These experiments show that spores of Bac. anthracis did not grow because of the destruction with nitrogen dioxide and not because the medium was unfavorable.

CONCLUSIONS

1) Nitrogen dioxide is a cheap intermediate product of the nitrogen industry.

2) Under the influence of strong bactericidal gas both vegetative and sporous forms of Bac. anthracis at a concentration of 0.5 g per 1 liter of air, at room temperature and normal atmospheric pressure are completely destroyed in 15 minutes.

3) It is worth testing nitrogen dioxide as a disinfectant of different objects in enclosed premises as well as in buildings infected with anthrax bacilli and their spores.